

SOME DEVELOPMENTAL CHARACTERS OF SPECIES
OF LYCOPERDACEAE

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INTRODUCTION

Most of the papers on the Gasteromycetes treat them from the standpoint of taxonomy; only a few deal with their morphology and physiology. Of these a few are limited to the Lycoperdaceae. The first reported study of Gasteromycetes was that of Corda (1842), who simply described the germination of spores of *Sphaerobolus stellatus* Tode. under natural conditions. Sachs (1855) was unable to germinate spores of *Crucibulum vulgare* Tul. Hoffman (1859) described the germination of the spores of the following species: *Lycoperdon echinatum* Pers., *Lycoperdon gemmatum* Batsch., *Bovista plumbea* Pers., and *Cyathus striatus* Willd. His later papers (1860a, 1860b) reported his failure to duplicate his first experiments. De Bary in 1863, although unable to germinate spores of *Phallus impudicus* L., gave us the first account of the development of *Lycoperdon* sp., *Bovista plumbea*, and *Geaster hygrometricus* Pers. (see De Bary, 1887). Pitra (1870) experimentally verified the previous observations of Corda on *Sphaerobolus stellatus*. Eidam (1875, 1876) produced an oidium-forming mycelium from spores of *Crucibulum vulgare* and *Cyathus striatus*. Hesse (1876) obtained a freely branching, oidium-producing mycelium from spores of *Cyathus striatus* which germinated in water after 18–24 hours. Brefeld (1877) grew mycelium of unnamed species of *Crucibulum* and *Cyathus* from single spores until fruit bodies were formed; these fruit bodies apparently developed in the usual way, but remained empty and failed to mature. Rehsteiner (1892) gave us the first critical study of species development, using *Lycoperdon gemmatum* Batsch., *Lycoperdon laxum* Bon., *Bovista plumbea* Pers., and *Geaster fornicatus* (Huds.) Fr. Rabinowitsch (1894) made a detailed study of *Lycoperdon depressum* Bon. Ferguson (1902a, 1902b) reported that she was unable to germinate spores of Gasteromycetes. Duggar (1901, 1905) grew tissue cultures of several species, but did not report the formation of any fruit bodies. Molliard (1909), using an entire peridiole of *Crucibulum vulgare*, eventually obtained the normal fruiting stage. He did not study the details of development. Cool (1912) reported her inability to germinate spores of Gasteromycetes. Cunningham published the details

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of the development of *Lycoperdon depressum* (1926) and the development of *Geaster velutinus* Morg. (1927). Lohwag (1926) discussed the phylogeny of Basidiomycetes in some detail and based his conclusions on the Lycoperdaceae upon the work of Rehsteiner. The writer published the details of spore germination in *Lycoperdon pyriforme* (Schaeff.) Pers. (Swartz, 1928). Gaümann and Dodge (1928) consider the whole matter of the classification of the Gasteromycetes as unsettled and subject to revision when our knowledge of it is increased.

The writer hopes that the following account of the morphological development of five hitherto unstudied species will contribute something of value to the yet unsolved problems of the Lycoperdaceae.

MATERIAL AND METHODS

The following species were selected for study: *Calvatia saccata* (Fr.) Lloyd, *Bovista plumbea* Pers., *Lycoperdon Wrightii* Berk., *Lycoperdon pulcherrimum* B. and C., and *Lycoperdon pyriforme* (Schaeff.) Pers.

Material representing all stages of development of the fruit bodies was collected, killed in Flemming's strong solution, and imbedded in paraffin in the usual way. Better penetration by the killing solution was secured if the air was exhausted from the fruit bodies by means of a suction pump. The exhaustion of air made the fixation of fairly large fruit bodies possible and also was advantageous in preventing injured tissues. However, many of the larger fruit bodies were cut longitudinally into two parts before fixing.

Sections were cut 5μ , 7μ and 10μ in thickness, but it was soon found that the sections 7μ thick were the most satisfactory. All the sections were stained with Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

Calvatia saccata

Description of young fruit body. Young fruit body at first equal, becoming depressed globose above, plicate below and abruptly contracted into a long stem-like base; the base thick and stout, often lacunose, nearly equal and tapering downward. Cortex a thin coat of small, persistent, meal-like granules. Attached to ground by thin white rhizomorphs.

Mature fruit body. Maximum size 12-15 cm. high, 6-8 cm. in greatest diameter. Upper globose fertile region supported by a rather stout, sterile, stem-like base 4-5 cm. in diameter, noticeably constricted below fertile portion. Outer surface roughened due to the drying of the terminal cells of the peridial hyphae, dingy yellow with, at times, a purplish tint. More or less regular depressions on sterile base,—abundant above and less frequent below. Basal part comprising greater part of mature fruit body. In section, base is composed of numerous rather closely formed cavities which become closer where base and rhizomorph are joined. Fruit body attached to

substratum by rhizomorphs at maturity. Spore mass brown, somewhat darker than the sterile tissue of the base. Opening by rupturing of peridium, which falls away, exposing spore mass; rarely a pore formed at apex. Large sterile stem remains attached to substratum long after spores have been scattered.

Rhizomorphs. The rhizomorphs are short, thin, white threads which taper greatly as they spread out from the fruit body. They are much thinner and branch less frequently than those of *Lycoperdon pyriforme* as they penetrate the soil and other débris present on the forest floor.

In longitudinal section (fig. 1, pl. 29) there are three layers: the two outer ones which are very distinct, and the third inner one, composed of hyphae which are interwoven with the hyphae of the layer surrounding it. The outermost layer or cortex is clearly defined, and is composed of thick, blunt, deeply staining threads which are interwoven considerably at the small end of the rhizomorph; here it makes up the greater part of the total diameter. The internal hyphae at the tip of the rhizomorph are compact, and many of them do not extend to the extreme tip; others which do are lost in the mass of cortex hyphae which covers the tip. These cortex hyphae extend directly into a mass of loosely connected, undifferentiated mycelium mixed with soil particles and other débris. The origin of these thick, heavy hyphae of the cortex is shown in longitudinal sections (fig. 1). They are not differentiated internally, and are so brittle that it is doubtful whether or not they are alive. Deposits of material, unevenly laid down within these threads, give them a darker color and a wavy outline. They can be traced to the threads of the subcortical layer, and are sometimes connected to living hyphae.

The subcortical layer, located just beneath the cortex, is composed of very closely interwoven hyphae which are, for the most part, parallel to the longitudinal axis of the rhizomorph. They have thin walls and do not stain deeply; this fact indicates a less active or sparse protoplasmic content. The hyphae of this layer are continuous with those of the central core.

The central core of the rhizomorph is composed of hyphae which, in part, originate in the subcortical layer. As the thinner hyphae of this outer layer pass into the center, they increase greatly in diameter. Their protoplasmic content is richer and they stain more deeply. The hyphae walls are also thicker. The nuclei, which are too small to be seen in detail, stain more deeply; several of these tiny nuclei occur in each cell. Septa occur occasionally and large vacuoles are present in the cells. Here and there in these hyphae are deposits of materials which may be in the form of large, angular, deeply staining crystals. The origin of this material is not clear, but the crystals are similar in structure to bodies reported by Conard (1915) in *Secotium agaricoides* (Czernaiev) Hollos. A few thinner hyphae may enter from the subcortical layer, grow for a short distance in the central region and then turn back into the region from which they arose. Such hyphae do

not stain deeply. Because these hyphae of the rhizomorphs of this species branch and interweave a great deal, the central region is not as compact as it is in other species.

Formation of the fruit body. It is extremely difficult to collect material to show the actual initiation of a fruit body. Such difficulty is due to the advanced stages of the fruit body when proper identification is possible. It is necessary to study the attached mycelium to find the initials, and very often after some of the fruit bodies have matured such young stages can be found no longer. However, in one instance material showing the initial stages was collected.

The first indication of a young fruit body is an enlargement on a rhizomorph (fig. 2). As this enlargement increases in size it becomes nodular. This nodular structure is directly connected to, and covered with the hyphae of the cortex of the rhizomorph. The heavy, thick, outer cortex hyphae are very conspicuous at this time, and are much more numerous where the now forming structure is connected to the rhizomorph. The interior hyphae of this initiated carpophore are much interwoven, and are simply continuations of the hyphae of the interior of the rhizomorph. They are thin-walled and rather frequently septate; the entire structure is homogeneous throughout (fig. 3). The young initial gradually becomes larger due to an increase in the number of hyphae in the interior. After a relatively short time, specialized hyphae appear which grow upward and branch outward toward the periphery. The peridium is initiated at the apex from these hyphae (fig. 4).

Peridium. The peridium is first distinguishable because of its reaction to stains rather than because of any noticeable morphological difference. The branching of the hyphae at first produces a loose arrangement of radial hyphae. The terminal cells of these hyphae which initiate the peridial layer soon become loosely connected and very irregular in shape. During its development the peridium is always more strongly developed in the apical region than toward the base. A second layer now develops just beneath the newly formed peridium. This layer is composed of loosely interwoven hyphae which are similar to the hyphae of the outer layer except they are not radial and do not stain as deeply. This layer does not differentiate further; instead it seems to serve as a base from which the outer hyphae of the peridium arise. As the fruit body expands the peridial hyphae become pseudoparenchymatous and this results in a much thicker layer.

The sterile base now elongates rapidly. At the same time the diameter of the fruit body increases considerably, more in the fertile than in the sterile portion. This increase results in a marked constriction at the connection of the fruit body and the rhizomorph. During the time of this expansion the sterile portion of the fruit body is covered by a layer of hyphae which are arranged quite differently than are the outer hyphae of the fertile part. These hyphae, although somewhat interwoven are chiefly parallel to the long axis. The layer thus formed is thin and never becomes pseudo-

parenchymatous. As the stem expands and elongates these hyphae bend outward, and a radial arrangement eventually results. This is not as clear cut as it is in the fertile part. Continued growth results in an extension of this radial arrangement to the basalmost part. The outward direction of the hyphae results in the formation of a collar around the fruit body at the junction of the fertile and sterile parts (fig. 5). The hyphae of the peridium dovetail with the outer hyphae of the sterile base in this region. Before the hyphae of the base turn outward, they grow upward for some distance; this upward extension prevents the radial arrangement of the component hyphae which is characteristic of the peridium proper. In the later stages of the formation of the sterile stem a deeply staining layer, situated just within the outer layer, is differentiated. The function of this layer is not clear, but because of the interwoven condition of the component hyphae it may perhaps function in contributing to the rigidity of the base. This layer, in some respects, is similar to the endoperidium formed by some of the lycoperdons.

At maturity, the peridium breaks up into irregularly torn pieces and falls away, thus exposing the powdery dry spore mass. When environmental conditions are unfavorable an apical opening, resembling the "pore" of the lycoperdons, may result. This is, of course, the exception, and the openings usually occur on the rounded surface rather than apically. Ordinarily the first ruptures of the peridium occur at the junction of the sterile and fertile portions.

In the above discussion the term peridium has been used to designate the structure covering the fertile globose portion. *There is no separation of the peridium into an endoperidium and an exoperidium*, as has usually been described. It seems advisable, however, to retain the term peridium rather than to present a new name for this covering. The presence of a one-layered peridium in this genus is significant and will be discussed later in the paper.

Gleba. In the earliest stages of fruit body development all of the tissue enclosed by the peridium is the gleba. In the initials of the fruit body, however, it cannot be distinguished as a morphological structure. The hyphae of the central part of the initial are intricately interwoven, thin-walled, many-septate and form a homogeneous mass. As increase in size takes place, the fertile portion becomes globose, and the interior hyphae become loosely interwoven.

Cavities soon appear in this rapidly enlarging body. They are irregular in shape and appear to be caused by the growth of the fruit body rather than by the dissolving of hyphae. They are formed first in the upper central region (fig. 6) and become larger; smaller ones appear toward the base. These cavities rarely have regular outlines at this stage because of the unequal proliferation of the hyphae. In this species the cavities are bounded by parallel hyphae which are more regularly arranged than those

in the lycoperdons. These parallel hyphae are most conspicuous when the cavities are being formed (fig. 7) and are the tramal hyphae which originate in the base of the fruit body, and wind about as they grow toward the apex. After the cavities become somewhat larger a rapid proliferation of hyphal branches takes place. These branches extend in all directions, and grow toward the outer limits of the cavities. Finally the ends of these branches form a regular palisade around the outer edge of the cavity. These hyphae again branch several times, and a number of hymenial units may result from one tramal hypha. This branching gives rise to a somewhat loosely interwoven zone between the trama and the hymenium. Rehsteiner (1892) has called this the subhymenial layer.

The cavities are not completely lined with basidia at one time; instead there is an uneven foundation of hymenium, and basidia are scattered around the edge of the cavities. Additional basidia are formed a little later and complete the hymenial layer (fig. 8). Tramal hyphae may give rise to the hymenium of several cavities as they wind from the base to the apex.

Occasionally stout hyphae, resembling those of the trama, pass from the hymenium on one side of a cavity, and become lost in the hymenium on the opposite side. Ordinarily such hyphae, quite common in this species, are not in the proper plane to be shown in one section of the fruit body. This may partly explain the opinions of other workers that cavity formation results entirely from the disintegration of hyphae rather than by unequal proliferation and separation of the glebal hyphae.

The cavities may become completely lined with hymenium before they show much increase in size. Increase in size takes place in two ways: (1) by the intercalary formation of new basidia and (2) by the coalescence of neighboring cavities. This coalescence occurs frequently. Two cavities, each having a continuous hymenium, are pushed closer and closer by the insertion of new basidia. Soon the tissue separating them begins to disintegrate and eventually completely disappears. For a short time the continuity of the hymenium is interrupted, but branches of the tramal hyphae soon give rise to additional basidia. This new hymenial layer is similar in appearance to the layer which had been formed earlier. There is no way to determine whether or not these new basidia form spores simultaneously with the old. New cavities may form between old ones, and become lined with basidia in the usual way.

Toward the base of the gleba undifferentiated tissue may still be seen. Cavities may be formed in this tissue even after those in the upper regions are completely mature. These are, however, ordinarily smaller than the first formed ones.

When the cavities have reached their maximum size, spores are produced—four on each basidium. At first they are small spherical swellings on the ends of the sterigmata; later the exosporium becomes thicker and roughened. The roughening is most conspicuous after the spores are mature. The

nuclei are too small to be seen satisfactorily during the process of spore formation.

Spore formation uses up a great deal of the trama and the subhymenial layer. A disintegration of these tissues now takes place. Many capillitium threads are formed. The moisture content is much reduced, and as a result the spore mass becomes dry and powdery. The peridium becomes soft and leathery, and spore dispersal takes place as described above.

Capillitium. The threads of capillitium arise from the growing tramal hyphae (fig. 9, pl. 30). From one of these hyphae a cell is cut off by a septum; the protoplasmic content of the resulting cell is greatly reduced, and crystalline material is deposited on the outside, making the outline irregular. At the same time granules appear on the inside. The deposits cause an increase in size and the structure becomes dark in color. Such threads occur in the peridium and in the sterile base. The formation of capillitium is not by the method suggested for *Lycoperdon depressum* by Cunningham (1926). Scattered threads of capillitium appear during the differentiation of the fruit body, but due to the disintegration and disappearance of the trama and subhymenial layer, they become more conspicuous. Capillitium evidently serves in the disposal of waste material rather than in spore dispersal as has been suggested.

Sterile base. Fruit body initials have no sterile base. A dense compact zone of hyphae which is connected to the interior of the rhizomorph gives rise to the base. The hyphae of this zone elongate considerably and curve outward. The interior of the resulting structure possesses cavities which are in every way comparable to those which develop in the fertile portion. Sometimes these cavities elongate laterally in the zone of transition between the fertile and sterile portions. Such cavity elongation does not occur in all fruit bodies, and it is only upon drying that the zone of separation becomes evident; it cannot be located in growing fruit bodies.

Threads of capillitium appear soon after the cavities are lined with hymenium. They first appear in the tissue between the cavities immediately after a period of great nuclear activity in the sterile hyphae which line the cavities. Since there is no disappearance of any tissue in the base, these threads remain scattered and are not particularly conspicuous.

Bovista plumbea

It is commonly known that fruit bodies of *Bovista plumbea* rarely occur in sufficient numbers, or in proper condition for a developmental series (Lloyd, 1902). In October, 1927, while passing through a pastured pine plantation east of Ann Arbor, the writer was fortunate enough to find representative fruit bodies of all ages growing under and among the fallen pine needles. The larger, more mature specimens were plainly visible as they emerged from the débris, but the smaller and younger ones were completely covered.

Description of fruit bodies. Young fruit body at first spherical, or depressed spherical, white; slightly areolate. Covered with a thin white cortex which early breaks up and falls away. Sterile base absent; fruit body weakly attached basally to substratum; easily separable.

Mature fruit body. Surface at first smooth, gradually becoming areolate with exoperidium easily separable and rupturing at boundaries of these areolae when drying occurs; and falling away in irregular pieces, thus exposing the soft, leathery, slate-colored exoperidium which persists as the tough papyraceous covering of the fruit body. Spore mass comprises the greater part of the plant when dry; microscopically composed of pedicellate spores mixed with capillitium. Opening, apical; sterile base, none. Fruit body much constricted at base, forming a thickened layer intermixed with soil particles, etc. Entire fruit body easily separable from substratum because of weak rhizomorphic connection.

Rhizomorph. Because of the ease with which fruit bodies may be separated from the substratum, it is very difficult to collect specimens showing a fruit body attached to a rhizomorph. The connection is so weak that occasionally fruit bodies become detached while they are still white and undifferentiated. When this happens they mature rapidly in the usual manner. There are two well developed layers of the fragile, slender, white rhizomorph. These layers are a central core, and an outer layer surrounding it; none of the blunt, dark cortex hyphae similar to those of *Calvatia saccata* are present. The sections studied were made of a rhizomorph still attached to a well developed fruit body.

Formation of the fruit body. The fundament of the youngest fruit bodies consists of an irregularly spherical, homogeneous mass of fairly large, much interwoven hyphae. Soil particles, old spores and other foreign matter cling to this mass of hyphae, and make cutting so difficult that badly torn sections often result.

Exoperidium. The initial of the fruit body described above soon becomes covered with a tangentially parallel layer of hyphae. The hyphae just beneath this covering branch profusely to form a zone of radial hyphae which are continuous with the hyphae of the outer layer. Accordingly the exoperidium is now composed of two different layers (fig. 11). The inner zone now becomes thicker and distinctly pseudoparenchymatous. The hyphae of the interior of the fruit body grow and cause the interior to expand, and the outer hyphae of this interior region enter into the formation of additional pseudoparenchyma in the inner zone of the exoperidium. The outermost layer gradually sloughs off with this increase in size, and is replaced by additional hyphae from the pseudoparenchyma. Sections show the two outermost layers to be easily separable from each other. At length the exoperidium is torn into ragged pieces which fall off. Ordinarily this starts at the apex, and gradually progresses toward the base.

Endoperidium. A continuous narrow zone of deeply staining hyphae

appears at the inner limits of the exoperidium. High magnification shows these hyphae to be loosely interwoven, and of greater diameter than those of the gleba. This zone becomes thickened, and the hyphae more compact due to the growth of new hyphae and the outward pressure of the developing gleba. Some of the hyphae on the outer edge are continuous with the hyphae of the exoperidium; while those toward the interior enter the gleba. However, this connection between the two peridia soon becomes weakened, and in my sections this weakened zone is demonstrated by the frequency with which the two peridia are separated. Very few sections could be made which were not separated at some place or other. When the endoperidium is well developed the hyphae composing it become darker, although the branches on either side may remain hyaline. The hyphae at the apex pull apart, and an opening for the escape of spores results.

Gleba. The differentiation of the gleba ordinarily begins very early. In this respect *Bovista plumbea* corresponds very closely to *Bovista nigrescens* Pers. (Rehsteiner, 1892). This differentiation is directly dependent upon environmental conditions and not upon the size of the fruit body. In this species as in *Calvatia saccata* glebal differentiation is more easily disturbed by unfavorable conditions than is the formation of the peridia.

Glebal differentiation is initiated in this species in much the same way as Rehsteiner (1892) found in *Bovista nigrescens*, and is similar to that of *Calvatia saccata*. A few points of difference should be pointed out. The hyphae of *Bovista plumbea* are generally coarser than similar hyphae in the species mentioned above; this is especially true of the tramal hyphae. There is no particular place for the formation of the first cavities, but they are usually situated in the central region with fewer toward the base. They appear promiscuously and are soon lined with hymenium; this is due to the branching of tramal hyphae. The formation of the hymenium takes place in a somewhat different manner than in any other species. The lateral or terminal branching of the tramal hyphae forms an umbel of secondary branches which have somewhat rounded ends. These grow toward the cavities and give rise to the basidia which line the cavity. Such groups of branches occur quite often, and as a result the subhymenial layer is thinner than in the other species.

As the fruit body increases in size new cavities appear as described for *Calvatia saccata*, and eventually the entire glebal region is a complicated network of cavities of various sizes. Such cavities coalesce quite commonly. The hymenium is similar throughout. No mature spores were observed until differentiation was almost complete. Rehsteiner (1892) saw mature spores very early in young fruit bodies of *Bovista nigrescens*. The spores in my studies were at first colorless, spherical and borne on short sterigmata. Later these sterigmata elongate, and remain attached to the spores when they are freed; the pedicel, therefore, is the sterigma which remains attached to the spore. Sometimes before detachment, sometimes after, the spore wall is thickened, and assumes a golden-brown color.

Capillitium. During later stages of spore formation the threads of capillitium become more conspicuous. They apparently are of hyphal origin much the same as *Calvatia saccata*, but the definite manner of their formation could not be determined.

Sterile base. The genus *Bovista* has always been separated from *Lycoperdon*, in part, by the absence of a sterile base. In the sections of *Bovista plumbea* examined in these studies a reduced sterile base was found, although it did not have the appearance of the sterile base of the lycoperdons, and cannot be rightly so called. In specimens not completely mature, the endoperidium and the exoperidium at the base are quite different from the peridia on the remainder of the fruit body. In some cases the sterile basal region was more manifest than in others. Where it occurs it is composed of closely interwoven vertically arranged hyphae. Here and there certain hyphae bend outward to the periphery, where they grow into the peridia. This mat of interwoven hyphae gives rise to a differentiated region in which there are no cavities, thus showing another difference from closely related genera. From all evidences this region of sterile tissue cannot be considered a true sterile base.

Dehiscence. The apical hyphae of the endoperidium become loosely arranged and an irregularly torn opening results. As the fruit body rolls from place to place the spores are freed. It is not uncommon to see old fruit bodies still containing spores one year after they have been formed.

Lycoperdon pulcherrimum

Description of young fruit body. Young fruit body at first subglobose to obovate. Covered with a cortex of long white convergent spines 3–4 mm. long; the spines connivent, becoming darker in age. Attached to substratum by well developed sterile base without deep rooting structure.

Mature fruit body. Fruit body obovate and covered with a well developed cortex of convergent spines except at the base. Spines becoming dark, somewhat curved, and falling away at maturity, exposing the smooth, silky endoperidium. Spore mass at first white, becoming brown in age. In median longitudinal section a marked constriction from the globose fertile region to the sterile base is visible.

The extreme shagginess of the spines of this species is approached by two species—*Lycoperdon echinatum* and *Bovistella pedicellata*.

Rhizomorph. The rhizomorphs are not strongly developed, and break away from the fruit body very easily at the time of collection. When they do remain attached they are often broken off, and lost during the process of killing and fixing. Such circumstances made it necessary to base the following remarks on examination of unstained material. The rhizomorphs are at first white, much branched and they extend into the soil some distance from the fruit body. Under the microscope two layers are visible, one a rather loosely connected layer of cortex hyphae having the same general

characteristics as those described for *Calvatia saccata*; the other layer is composed of very thin hyphae which run parallel to the longitudinal axis. Neither layer is strongly developed.

Development of fruit body. Fruit bodies of this species arise in much the same way as in *Calvatia saccata*; either laterally or terminally from a rhizomorph or a branch of it. Sections through the youngest available fruit bodies show a compact, homogeneous mass of closely interwoven hyphae. The peripheral hyphae become radially arranged very early and form the initial of the exoperidium.

Exoperidium. Radial hyphae appear simultaneously over the periphery of the fruit body. This differs somewhat from the condition in *Lycoperdon pyriforme* and *Calvatia saccata*, where no peridium is formed around the sterile base. These hyphae, at first, have a uniform diameter, and their tips form a compact, slightly interwoven layer which completely encloses the fruit body. The walls of the hyphae are thin, and the protoplasmic content of the cells seems somewhat reduced. As in *Bovista plumbea*, the inner part of the layer becomes pseudoparenchymatous after the endoperidium is formed.

Gradually the interior of the fruit body expands, and some of these interior hyphae proliferate into the developing exoperidium and assume the characteristic radial arrangement. Due to this internal expansion the outermost hyphae of the exoperidium separate, and fissures appear between groups of them; this gives rise to conical groups of cells which later become irregularly shaped because of their loose connection. With additional expansion the fissures become deeper and extend into the pseudoparenchyma. The exoperidium is naturally very thick, and the fissures are correspondingly deep. The conical arrangement of hyphal cells between the fissures is really due to the spines which are so characteristic of this species. It follows then that the longest spines simply result from the deepest fissuring.

After these long spines have formed, the entire outer layer is very easily disturbed during cutting, and tends to separate from the endoperidium. The cells composing the tips of the cones are not as easily separable from each other as they are in *Lycoperdon pyriforme*; this indicates a closer relationship of these cells, due, at least in part, to the somewhat interwoven condition of the hyphae from which they are formed. This partially explains the curved tips and convergence of the spines. After the endoperidium is formed the fissures keep pushing in until they reach it. The endoperidium is, however, never split. In later stages the cortex of spines falls off and exposes the endoperidium.

Endoperidium. Shortly after the differentiation of the distinct outer layer which gives rise to the exoperidium, a zone of thin, loosely interwoven hyphae appears some distance beneath it. This zone completely encircles the interior; the component hyphae do not stain deeply and have a thin wall. The branching of the original hyphae of this layer, as well as the entrance of new hyphae from either side, causes it to increase in thickness. The

hyphae on the outside of this layer are continuous with the hyphae of the exoperidium; and those on the inside are continuous with the yet undifferentiated gleba. Just within this newly formed endoperidium there is a growth zone which remains unchanged until after the gleba has differentiated.

Gleba. The gleba is formed as in *Lycoperdon pyriforme* and *Calvatia saccata*. Cavity formation, however, begins much earlier, and the first ones appear about the same time the endoperidium is laid down. They appear between rapidly growing hyphae and at first are irregular in outline. They are formed promiscuously throughout the upper central part of the gleba, and continue to form until the greater part of the gleba is used up. Their increase in size takes place as in *Calvatia saccata* and *Bovista plumbea*. The hymenium which eventually lines the cavities arises from a very poorly developed trama, whose component hyphae show only a very slight tendency toward a parallel arrangement. Because of this the subhymenial layer is weakly developed; the hyphae of this layer resemble those of the trama. There is no noticeable transition from one tissue to the other. Here and there, as in *Calvatia saccata* and *Bovista plumbea*, large, thick-walled hyphae pass through the cavities from one hymenium to the other.

The trama ordinarily disintegrates during the formation of spores, and mature spores may be seen in the cavities before differentiation is complete. This corresponds to *Bovista nigrescens* as described by Rehsteiner (1892).

Capillitium. Capillitium is formed in this species exactly as described for *Calvatia saccata*.

Sterile base. The sterile base was poorly developed in all the fruit bodies studied. There is no definite line of demarcation marking the transition from fertile to sterile tissue. Cavities are formed in the sterile base, but are not lined with a palisade of sterile hyphae as in *Calvatia saccata*. The hyphae making up the base are thin and much interwoven; they originate in the central hyphae of the rhizomorph, pass upward through the sterile base into the fertile region, and there they become interwoven with the hyphae of the peridia.

The outer covering of the sterile base is a layer of radial hyphae. This covering layer is not as thick here as around the fertile portion (fig. 12). The splitting of this layer, therefore, does not give rise to fissures as deep as those near the apex, and as a result a thick cortex of spines is not produced.

Dehiscence. When the fruit body has matured the apical hyphae of the endoperidium become rearranged and are pulled apart. This gives rise to the apical pore through which the spores escape.

Lycoperdon Wrightii

Description of young fruit body. Fruit body spherical, rarely oval; irregularly compressed due to close association with neighboring fruit bodies; ordinarily sessile, rarely with a short stalk. Cortex very rough, spiny; spines

thickly clustered, bending together at apex; becoming dark very early, and imparting a dull yellowish color to the young fruit body.

Mature fruit body. Mature fruit body covered with a thick spiny exoperidium; exoperidium breaking into rough, ragged, unevenly torn pieces which are readily separable from the endoperidium when dry. Endoperidium papyraceous, very tough, persistent, at first orange colored. Spore mass olive in color and occupying the greater part of the fruit body. Exteriorly no definite zone of transition from the fertile to sterile portion. Sterile base present, very poorly developed, and composed of large, unevenly formed cavities; persistent, often remaining attached after spores have been scattered.

Rhizomorph. It was necessary to examine free hand sections of the rhizomorphs, as they are so delicate and easily separable from the fruit bodies that they were invariably lost during dehydration and infiltration. They are thin, branched, and covered with outgrowths of short white hyphae. In all other respects they correspond closely to the rhizomorphs of *Lycoperdon pulcherrimum*. The cortex of dark-colored, blunt hyphae is not as strongly developed in this species as in *Lycoperdon pyriforme* and *Calvatia saccata*.

Development of fruit body. Adhering soil particles make it extremely difficult to cut satisfactory sections of fruit body initials. A few sections were obtained which were not badly torn, and which showed the structure of this mycelial mass very well. It is loosely interwoven and composed of fairly large hyphae throughout. In the youngest fruit bodies, the outermost hyphae are arranged radially and extend around the fruit body as far as the base. The hyphae at the base are more compact, and arise directly from the rhizomorph. These hyphae grow upward and bend toward the periphery.

Exoperidium. The radial hyphae mentioned above form the first exoperidial layer. These hyphae are coarse, and have a thin wall. They branch frequently and soon become somewhat interwoven. As development continues the exoperidium becomes evenly thickened except at the base. During this thickening the hyphae become more interwoven, and additional hyphae enter from the undifferentiated interior. At this stage the exoperidium composes the greater part of the total diameter (fig. 13). Later the exoperidium becomes pseudoparenchymatous. The gleba now differentiates rapidly, and since the outer layer of the exoperidium does not keep pace with this internal expansion, fissures appear, and as a result spines are formed. This process is similar to that in *Lycoperdon pulcherrimum*. In cross section the fruit body resembles *Bovista plumbea* except that the latter does not have spines, but instead the outermost hyphae are arranged tangentially parallel. There are places in the exoperidium of *Lycoperdon Wrightii* where the hyphae are loosely arranged (fig. 14); the areas indicate the cracks which are formed later before the exoperidium falls off. Areas like these are not formed in *Bovista plumbea*; otherwise the two exoperidia are somewhat similar. After the exoperidium falls off at maturity, the endoperidium remains as the covering for the spore mass.

Endoperidium. The formation of the two peridia is simultaneous. The endoperidium, at first, is simply composed of a few thin-walled hyphae which are nearly parallel, and are tangential. Gradually it becomes more interwoven, compact and thickened. In this case, as in the others previously described, the endoperidial hyphae are continuous with the exoperidial hyphae on the outside and with the glebal hyphae toward the interior. This layer of hyphae extends completely around the fruit body, and forms a *definite line of separation between the globose fertile region and the more constricted sterile base*. At maturity an apical opening is formed.

Gleba. The formation of the gleba is similar to that of *Calvatia saccata*. A complicated network of cavities results; these become lined with hymenium which arises from a poorly developed trama. This trama is similar to that of *Lycoperdon pulcherrimum*. Spore formation and subsequent differentiation takes place as in the preceding species.

Sterile base. An unusually small part of the tissue in this species is sterile, and this is confined to the base. The hyphae of the basal zone show no change until after the other tissues are almost completely formed. Cavities are present here as in other species with sterile bases, but they are correspondingly fewer in number. As has already been pointed out, the sterile base is separated from the fertile part by the continuous endoperidium. The hyphae of this region appear white in mass. As in the preceding species the base remains attached to the ground after the spores have been scattered.

Dehiscence. At maturity an apical opening is formed as in the preceding species. The spores are freed through this apical opening.

Lycoperdon pyriforme

Description of young fruit body. Fruit body pear-shaped from the beginning; covered with a cortex of minute, fasciculate, subpersistent spines evenly scattered over the globose fertile portion. Sterile base fairly well developed in the younger stages; attached to woody substratum by a long, white, fibrous mycelial strand.

Mature fruit body. Fruit body pear-shaped with a fertile globose head supported by a fairly well-developed sterile base; partially covered with dirty whitish spines. Pore formed apically. Gleba changing from white to yellowish brown at maturity. At first there are two distinct peridia, but only one ordinarily is persistent until fruit body is fully mature. No definite line of separation between the brown spore mass and the sterile base. Sterile base attached to a well developed rhizomorph. Rhizomorph ordinarily on wood or in ground rich in decaying wood.

Rhizomorph. The rhizomorphic strands, which are more fully developed in this species than in any other, are made up of tough cords of white mycelium. They commonly grow on wood or in soil rich in decaying wood. A relatively large section of the rhizomorph ordinarily remains attached to the fruit body. In longitudinal section the rhizomorph has three layers.

The outermost layer is the cortex, which is composed of large, blunt, unbranched, dark-colored, loosely interwoven hyphae which make up one-fourth to one-third of the total diameter. These hyphae have no cross walls and are devoid of protoplasm. They arise from the hyphae of the layer just beneath, and are somewhat interwoven with them where the layers unite. The uneven thickening of their walls gives them a wavy appearance. These cortex hyphae have a protective function, and are fairly easily disturbed during handling. A much thicker layer of these hyphae covers the tip of the rhizomorph.

The subcortical layer is composed of thin-walled, sparsely septate, and rarely branching hyphae which run parallel to the longitudinal axis. They are uniform in shape and size, and are but little interwoven. They also contain a reduced amount of protoplasm. Such compact, long-celled hyphae doubtlessly strengthen the rhizomorph.

The central core is composed of little branching, multinucleate hyphae which are very rich in protoplasm. These hyphae stain deeply and sometimes there is a continuity of protoplasm between adjacent cells of the same hypha. There are somewhat larger, deeply staining bodies of two shapes within the central cells. These bodies are either spherical or cubical and stain very deeply. Similar bodies were called protein crystals by Conard (1915) in rhizomorphs of *Secotium agaricoides*.

At the tips of the rhizomorphs the two inner layers converge, and stain more deeply, thus indicating a richer protoplasmic content. The tip is covered with a compact, thick layer as in *Calvatia saccata*.

Formation of fruit body. Fruit bodies arise either laterally from rhizomorphs or terminally from branches of them. Obtaining material to show the first initial stages is difficult and uncertain. The first evidence of the new fruit body is a modification of the hyphae of the rhizomorph. Such groups of modified hyphae become interwoven, expand and give rise to nodular swellings. Expansion and enlargement of these hyphae continue until the nodular structure protrudes slightly on the rhizomorph as in *Calvatia saccata* (fig. 2). A more rapid expansion of the interior of this structure now forces the exterior hyphae outward, and the increased internal pressure causes the cortex to become thinner, but the cortex hyphae are still continuous with the outer layer of hyphae of the rhizomorph. The young fruit body, which is at first visible only through a hand lens, now becomes visible to the naked eye. With the exception of the adhering cortical hyphae the young fruit body has remained homogeneous throughout; it is composed of intricately interwoven hyphae rich in protoplasm. Differentiation now takes place rapidly.

Exoperidium. The outermost hyphae at the apex of the initial become loosely arranged, and, as expansion continues, a layer of radially arranged, thin-walled hyphae gradually appears. At first this change is limited to a very narrow apical region. This layer becomes the exoperidium, which

eventually covers the entire fruit body, and it is always more strongly developed at the apex than elsewhere. For a relatively short time it remains intact, but the outermost cells gradually lose their protoplasm, become irregular in shape and very loosely connected. The surface growth ceases and the outer layer becomes fissured, giving rise to the conical warts attached to the endoperidium (fig. 15). These warts are made up of loosely connected cells which may fall off, and as a result the warts do not keep their regular conical shapes. The fissures penetrate radially, and eventually the layer of pseudoparenchyma which makes up the interior of the exoperidium is involved. New exoperidial hyphae form continually by the outward branching of the hyphae of the endoperidium. By this time the upper portion of the fruit body is covered by the developing exoperidium, which never completely extends to the base. The hyphae of the exoperidium become interwoven with the outermost hyphae of the base. This interweaving of the hyphae forms a rather conspicuous shoulder around the fruit body. Due to the splitting of the outer layer at the exoperidium, it gradually falls off until at last nothing remains of it except a layer of pseudoparenchyma. This layer also gradually becomes thinner at maturity.

Endoperidium. Shortly after the exoperidium is initiated at the apex, a second layer, recognizable by a deeper staining reaction, is formed. At first this layer is confined to a rather restricted zone at the apex, but it gradually extends downward toward the shoulder. The component hyphae are parallel at first, but due to rapid branching they soon become interwoven, and assume a tangential position. Some of these hyphae turn outward and become a part of the exoperidium; others turn inward and become a part of the gleba. This layer of hyphae just described is the endoperidium. It gradually becomes more compact because of the outward pressure exerted upon it by the developing gleba, and persists in this condition until the fruit body matures.

During the later stages of development, after the sterile base has been delimited, a growth zone appears in the region where the peridial hyphae meet the outer hyphae of the sterile base. From this zone new endoperidial hyphae are formed. These newly formed hyphae soon become interwoven with the original endoperidium, and gradually become compact. This gives rise to the distinct endoperidium which surrounds the sterile base at maturity.

Gleba. The gleba is at first composed of a compact mass of intricately interwoven hyphae. These hyphae are rich in protoplasm, and are similar throughout. Rather early in the development of the fruit body the hyphae of the interior apical region become loosely interwoven, and two distinct types of hyphae appear. One type is very coarse and always originates in the basal region, bends outward and grows toward the periphery. In some cases these hyphae extend to the endoperidium. The other type of hyphae is smaller and more nearly resembles the hyphae of the gleba initial; they are multiseptate, freely branched and thin-walled. The larger hyphae are only occasionally septate and branch very rarely.

Cavities are first formed in this region near the apex, and later gradually appear toward the base. *These cavities are really interspaces between hyphae and arise by mechanical splitting.*

While the cavities are being formed a strongly developed network of bundles of parallel hyphae appears in the interior. These bundles are made up of four or five hyphae which by increase form the trama. These tramal hyphae branch, and the branches grow toward and into the periphery of the cavity. Here they form a palisade layer. The development of this layer is uniform in some places, but the periphery of the cavity is not lined with these cells at exactly the same time. The subhymenium lies between the hymenium and the trama, and is a tissue of loosely interwoven hyphae which are also branches of the trama.

While the hymenium is being formed a new layer of tissue is differentiated just within the endoperidium. It consists of much branched, interwoven hyphae. There are no cavities in this tissue at first, but later they appear.

New cavities with hymenium arise in many places between older ones. They sometimes fuse when two are close enough together. This fusion, or anastomosis as it has been called (Rehsteiner, 1892), results from the disintegration of the hyphae between. This is the same in all the species studied. After the greater part of the gleba has been used up, cavities begin to form in the secondary tissue just within the endoperidium. The manner of formation is somewhat different here. Basidia appear first on the side of the cavity toward the interior. This condition persists for some time, and is similar to that described for the early stages of cavity formation in *Hysterangium clathroides* as described by Rehsteiner (1892). Gradually, however, the hymenium spreads over the outer sides of such cavities.

Spore formation. The basidia show the effects of lateral pressure by flattened sides, but the ends are still rounded. A short time after the basidia appear, a septum forms at the base. The basidia are usually four-spored, and the first spores appear on the first-formed basidia. In some of the spores nuclei may be seen readily; in others they cannot be seen. Cunningham (1927) explains this situation by assuming that nuclei are present but that they are not stained.

After the spores are formed a rapid disintegration of all the remaining gleba hyphae and basidia takes place, leaving nothing except spores, capillitium and a few scattered crystals. This remaining mass is surrounded by the endoperidium which has become tough and paper-like.

Capillitium. Capillitium is formed in this species as in *Calvatia saccata*. It is very definitely of hyphal origin, and becomes conspicuous only after most of the gleba has been used up in spore formation.

Sterile base. The hyphae in the basal region remain comparatively inactive during the differentiation of the gleba, and have an upright position parallel to the long axis of the fruit body. An elongation accompanied by an increase in thickness takes place in this region. Prior to this development

the reduced exoperidium of the base has not been differentiated, and the enclosing hyphae are similar to the outer hyphae of the rhizomorph. A few cortex hyphae adhere to this layer, but as elongation continues an exoperidium-like tissue is formed near the point of union of the fertile and sterile tissue. This becomes the outer covering of the mature sterile base, but it is much reduced when compared to the exoperidium of the fertile part. In addition it does not extend very far down along the side of the sterile base. In the interior of this region, cavities which become lined with basidia are formed. Such cavities here are longer and do not anastomose freely, and the basidia are more regular. In all cases, however, these cavities are not completely covered by basidia and they do not stain as deeply as do the spore-producing basidia of the fertile cavities. The layer from which these hyphae arise is ordinarily poorly developed. Toward the lowest part of the base the smallest and most poorly developed cavities are formed. At maturity such cavity formation has continued until they are present almost to the region of attachment to the rhizomorph. These later-formed cavities are rarely lined with basidia. There is no definite line of demarcation between the fertile and sterile parts of the fruit body; no morphological explanation can be offered for the columella as it is seen in mature specimens.

Dehiscence. As the tissues of the gleba disintegrate, the endoperidium becomes modified at the apex. The hyphae become much looser and the remaining tissue becomes much thinner here than elsewhere. In this small area the hyphae are eventually pulled apart, and in this manner the pore is formed. Just why it should take place at this point is not clear.

DISCUSSION

Atkinson (1914a, 1914b, 1914c, 1916) in his studies of certain agarics pointed out the advisability of studying the development of closely related species of fungi in order to get a complete working knowledge of their relationships. His results showed that even though species bear a close resemblance externally, their methods of internal development are not always the same. The same fact holds true in our study of the Lycoperdaceae; similar species may develop quite differently.

Bonorden (1857) and Tulasne (1842) recognized that unless fruit bodies have had fairly favorable conditions for development, considerable difficulty may be encountered in placing them in their proper taxonomic positions. Rehsteiner (1892) and the writer (Swartz, 1929) have found that unfavorable environmental influences affect the internal development much more than the external. It seems well, in view of these facts, to state that only healthy and normally developed fruit bodies should be used as the basis for any study whatsoever. For that reason the utmost care was used in selecting the material for this work.

Lloyd (1902), Rehsteiner (1892), and Coker and Couch (1928) have

stated that the peridium of species of Lycoperdaceae may be separated into two layers at least—namely, an inner layer, which is the endoperidium, and an outer layer, the exoperidium. In the genus *Geaster* three layers are ordinarily present. From the foregoing developmental studies, it is evident that the situation in *Calvatia saccata* is different. The peridium in this species is composed of only one layer; there is, in addition, relatively little sculpturing on the exterior. This layer is, without doubt, the endoperidium; and the sculpturings and markings occupy the position ordinarily taken by the exoperidium of other species. Such a modification seems logically of sufficient importance to substantiate a separation of the genus *Calvatia* from the genus *Lycoperdon*. In *Lycoperdon pulcherrimum*, where the youngest fruit bodies resemble *Calvatia saccata* quite closely, two very clearly defined layers develop. The exoperidium is especially well developed, and is covered by a cortex of long convergent spines. The differentiation of the exoperidium is followed later by the formation of the endoperidium. The endoperidium is never fissured, even after all of the tissue of the exoperidium becomes involved. The exoperidium of *Lycoperdon pyriforme* is, however, simpler than that of the foregoing species. It is similar for the most part, but is not so thick and has a looser arrangement of the cells making up the spines. The looser arrangement of the cells allows the spines to behave in like manner. In this species the pseudoparenchyma is not necessarily fissured; it sometimes persists until maturity as an outer covering for the endoperidium. The production of a cortex of spines in *Lycoperdon Wrightii* is similar to the preceding, but the peridium is quite different. The shedding of this outer layer corresponds more closely to the same process in *Bovista plumbea*, where the exoperidium reaches the highest development of any species studied. In *Bovista* areolae are formed which serve in the same capacity as the weakened areas in *Lycoperdon Wrightii*. The exoperidium of each species ruptures in these specialized areas. In addition to the rupturing in these areas, the two peridia often separate here, too. This separation occurs only after the formation of pseudoparenchyma, and it should be remembered that this tissue may be pulled apart more easily than any other tissue of the fruit body. It does not seem a very great step from this arrangement of peridial layers in *Bovista* to the three-layered peridium which Rehsteiner (1892) has described in *Geaster*. In *Geaster* the separability of the layers is quite evident; the layers do not necessarily fall away; but on the other hand, they persist and become reflexed. From the evidence at hand it appears that the exoperidium in *Calvatia saccata* has become so reduced that the adhering meal-like granules are the sole remains of it. No structures are formed in this species which can be considered homologous with the spines of *Lycoperdon pulcherrimum*, *Lycoperdon Wrightii*, or *Lycoperdon pyriforme*. These spines apparently should be considered homologous with the outer layer of tangentially arranged hyphae in *Bovista* and corresponding to the outermost layer in *Geaster*, where the outer layer reaches the maximum development in the Lycoperdaceae.

The endoperidium of *Calvatia saccata* is the outer rind, and it has been developed strongly enough to assume the function of the exoperidium. In *Lycoperdon pyriforme* the endoperidium is initiated at the apex and develops until it completely encompasses the fertile globose portion and becomes interwoven with the exterior hyphae of the sterile base. *Calvatia saccata* does not show this relationship to the same degree shown in every species of *Lycoperdon* studied. In every other species the hyphae of the endoperidium are continuous with the regions on either side; this connection may be rather loose because of the separability of the pseudoparenchyma. The compactly interwoven hyphae in the later stages of the development of this tissue explain the tough, leathery consistency of the mature endoperidium.

In *Bovista plumbea* and in *Lycoperdon Wrightii* the endoperidium is one of the first structures laid down, and it soon extends completely around the fruit body. This continuation of the endoperidium reminds one of the "grenz-linie" described for *Lycoperdon depressum* by Rabinowitsch (1894). This is not present in any of the other species studied.

The gleba, during differentiation and at maturity, is similar in all species studied. In every species the hymenium arises from a trama. In *Lycoperdon pyriforme* and *Calvatia saccata* the trama is well developed and consists of organized groups of branching, parallel hyphae. The trama is not so well developed in *Bovista plumbea* and it is very poorly formed in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. There is a definite layer of subhymenial hyphae in *Lycoperdon pyriforme* and *Calvatia saccata*; this layer is not well developed in *Bovista plumbea* and is inconspicuous in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. In the last two species it cannot be distinguished from the trama.

Cavity formation is similar in all species except *Bovista plumbea*, and the coalescence takes place similarly in all species. Rehsteiner (1892) pointed out that the basidia of the then investigated species of *Lycoperdon* and *Bovista* were similar. In our species this remains true. Spore formation is similar throughout the range of species. Rehsteiner also pointed out that glebal differentiation in *Geaster fornicatus* is similar to that of the lycoperdons and bovistas.

The formation of capillitium in our species of Lycoperdaceae is much different from capillitium formation in Myxomycetes as reported by Harper (1914) and Harper and Dodge (1914). The threads are often somewhat similar in appearance, but in the Lycoperdaceae they arise from growing hyphae. The workers mentioned above do not find this to be true in Myxomycetes. In all of our species capillitium formation is similar. No connection between capillitium and peridium was found in any species, as indicated by Rehsteiner (1892) and Cunningham (1926). Capillitium in the Lycoperdaceae ordinarily appears first in the trama. As the trama is used up in spore formation and subsequent disintegration, these threads become more conspicuous. Their irregular outline, as well as the thickening

of the wall and the occurrence of crystalline deposits with them, has led to the conclusion that they are more associated with the disposal of waste products than with the dispersal of spores. Rehsteiner (1892) found that similar deposits in related species were calcium oxalate. He found these crystals more or less regularly arranged along capillitium threads, particularly in *Bovista nigrescens*. The same is true for the species described in this paper. Cunningham (1927) stressed the appearance of capillitium threads in the region of the endoperidium. Such threads occur in similar locations in our species, but not in sufficient numbers to deserve special mention; instead, they occur scattered throughout the gleba. In *Bovista* the capillitium consists of star-like groups of hyphae. Capillitium formation reaches its highest development in *Geaster hygrometricus*, according to De Bary (1887). In this species the threads arise from hyphae originating in the columella; and after differentiation is complete, a definite skeleton or network is visible. This arrangement, according to De Bary, is somewhat similar to the receptaculum of certain phalloids, and is considered as functioning during spore dispersal. This has led to the opinion that capillitia are effective during spore dispersal. It seems logical, in view of the above similarities in structure and formation, to consider capillitium and the receptaculum homologous structures whose functions have been somewhat modified. Aside from this, little can be found in the gleba upon which relationships can be based.

The sterile bases of different species show different degrees of development. In some species, as in *Calvatia saccata* and *Lycoperdon pyriforme*, the sterile base is relatively large; in other species, as in *Bovista plumbea*, practically no sterile base is developed, while in *Lycoperdon Wrightii* there is a small, poorly developed one. The sterile base, although not as sensitive to environment as the fertile portion, may be somewhat affected by it. Ordinarily the bases do not develop as early as the fertile part; instead, they remain relatively inactive during the early developmental stages. When they begin to differentiate, elongation and increase in diameter take place simultaneously, and by the time of spore formation, the sterile base has reached its specific proportions. According to De Bary (1887), the columella of *Geaster* is really a much reduced sterile base which occupies a relatively small area in the fertile region and often disappears at maturity. From *Calvatia saccata* through *Lycoperdon pyriforme*, *Lycoperdon pulcherrimum*, *Lycoperdon Wrightii*, and *Bovista plumbea* there is a gradual reduction in the size of the sterile base until we find it either absent or reduced to the internal columella in the higher Lycoperdaceae as shown by the geasters.

Fruit body modification for spore dispersal varies in different species. In *Calvatia saccata*, with only one peridium, this outer layer usually breaks up into irregular pieces and falls away. Occasionally in this species an apical opening is formed, but this is not characteristic as it is in species of *Lycoperdon*, *Bovista*, and *Geaster*. Although the details of pore formation are very hard to detect, the rearrangement and thinning out of the hyphae at

the apex were seen to take place in the manner described by Rehsteiner (1892). In *Bovista plumbea* and *Lycoperdon Wrightii* the exoperidium falls away before pore formation takes place, but this is not necessarily true in all species. The highest type of pore formation is seen in the genus *Geaster*, where the pore character is of great taxonomic importance. *Calvatias* having no pores may, therefore, be considered the simplest, with the lycoperdons and bovistas occupying intermediate positions.

Previous workers have failed to examine rhizomorphs in any great detail. Of the species studied in this paper, *Lycoperdon pyriforme* has the most highly specialized rhizomorph. In this species they are stout, much branched structures. The internal structure strongly resembles that of *Calvatia saccata*, where they are not nearly as strongly developed. The connection of the rhizomorph and fruit body is particularly weak in *Bovista plumbea*; this explains the ease with which these fruit bodies become "tumblers." The other two species treated in this paper are as yet incompletely investigated. The habitat of these fungi usually plays a very important part in determining the nature of the rhizomorph. This is illustrated very well by *Lycoperdon pyriforme*, which is ordinarily collected on wood in some stage of decay; the rhizomorph in this species sometimes extends more than a foot away from the fruit body. In the ground-inhabiting species such well developed rhizomorphs are seldom found; instead, they are smaller and confined to a more restricted zone in the substratum. In *Geaster*, as described by Rehsteiner (1892), no rhizomorph is developed; instead, the fruit bodies are attached only by weak tufts of mycelium. Considering the growth habits of *Geaster*, this modification seems much to its advantage in allowing the fruit body to be raised up at maturity. This reflexing as well as the ease of detachment from the substratum should greatly aid in spore dispersal. These characters combined with the others mentioned above seem to indicate the advanced position of the geasters in the family Lycoperdaceae.

SUMMARY

1. The detailed morphological development of the following species was studied: (1) *Calvatia saccata*, (2) *Lycoperdon pyriforme*, (3) *Lycoperdon pulcherrimum*, (4) *Lycoperdon Wrightii*, (5) *Bovista plumbea*.

2. The peridium of *Calvatia saccata* was found to consist of only one layer. This finding is at variance with the reports of others who describe a two-layered peridium for this genus.

3. The finding of a definitely one-layered peridium adds another morphological character to those ordinarily accepted for *Calvatia*. This character is sufficient to establish the validity of the genus *Calvatia* as distinct from *Lycoperdon*.

4. The investigated species of *Lycoperdon* all show clearly a two-layered peridium.

5. A modified sterile region was found at the base of *Bovista plumbea*;

the fruit body, with the exception of the peridia, has been considered completely fertile. The very weak connection of this sterile tissue to the rhizomorph explains the ease with which the fruit bodies may be separated from the substratum.

6. The rhizomorphs of the investigated species are not homogeneous throughout; they are composed of two or three distinct layers of hyphae. This depends on the species. Ordinarily there are three layers—a cortical layer, a subcortical layer, and a central core.

7. The tissue of the interior of a fruit body initial is very similar to the interior tissue of the rhizomorph and arises directly from it, and the tissues of the fruit body differentiate from this.

8. The spines, warts, and other exterior markings of the fruit bodies arise simply from the failure of this outer layer to develop as rapidly as the interior. Uneven drying as well as continued internal expansion causes these exterior roughenings to fall off.

9. Cavities arise during differentiation as interspaces between hyphae. This is due to a mechanical splitting rather than to a dissolving of original hyphae.

10. The fertile and sterile regions are very rarely separated by definite morphological structures in the fruit bodies investigated. *Lycoperdon Wrightii* is the most noteworthy exception.

11. Capillitial threads develop from growing hyphae, and serve as reservoirs where waste materials accumulate.

12. There is a remarkable similarity in the differentiation of tissue in the five species included in this paper.

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EXPLANATION OF PLATES

PLATE 29

Fig. 1. Longitudinal section of a rhizomorph, showing the heavy, deeply staining hyphae of the cortex, the parallel subcortical layer and the central core.

Fig. 2. Showing a section of a rhizomorph and the attached young fruit body still appearing as a nodule on the outside of the rhizomorph.

Fig. 3. Later stage of figure 2 showing the heavy, dark threads still adhering to the outer edge of the fruit body. The structure is still homogeneous.

Fig. 4. A group of the peripheral hyphae at the apex which have begun to arrange themselves in the characteristic manner of the peridium.

Fig. 5. The enlarged collar-like region at the point of union of the sterile and fertile regions of the fruit body. Also characteristic arrangement of peripheral hyphae of the base.

Fig. 6. A very young fruit body in which the peridium is well developed and cavities are beginning to appear between loosely interwoven hyphae. Note that the vertical hyphae of the base are still attached to the hyphae of the rhizomorph.

Fig. 7. A segment of the hymenial layer of a cavity, showing the compact arrangement of the hymenium, and the loose arrangement of the subhymenial hyphae. Note the comparatively abrupt connection to the parallel hyphae of the trama.

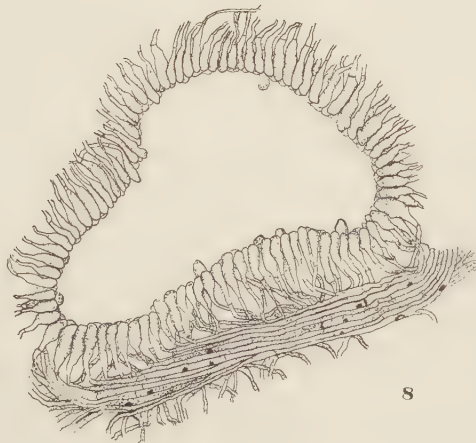
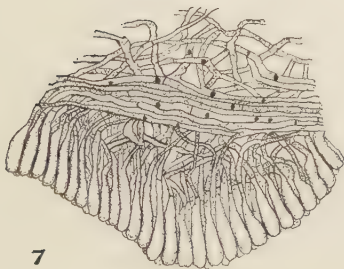
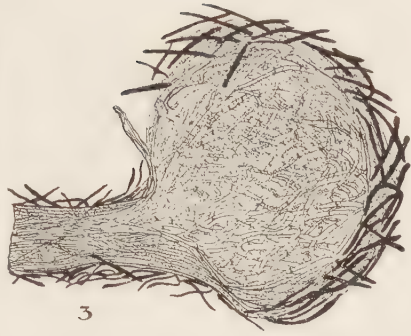
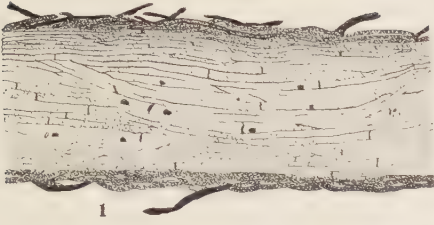
Fig. 8. A highly magnified cavity showing the relation of the hymenial layer to the parallel tramal hyphae and showing the intercalation of newly formed basidia; the basidia are not mature.

PLATE 30

Fig. 9. A portion of the hyphae between cavities showing the manner in which capillitium is formed from growing hyphae.

Fig. 10. The basal connection of *Bovista plumbea*. The central hyphae are loose, and this accounts for the breaking away of the fruit body in the center.

Fig. 11. A section of the peridia of *Bovista plumbea* showing the tightly woven endoperidium, connected to the pseudoparenchymatous inner layer of the exoperidium. Also note the tangentially parallel arrangement of the outermost hyphae.



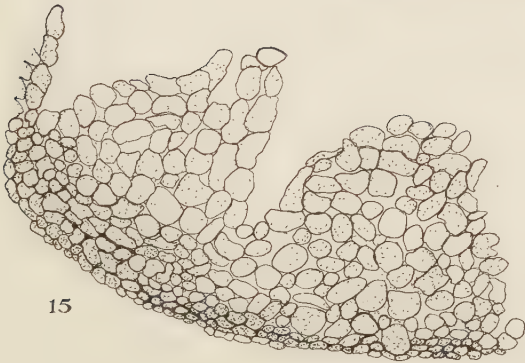
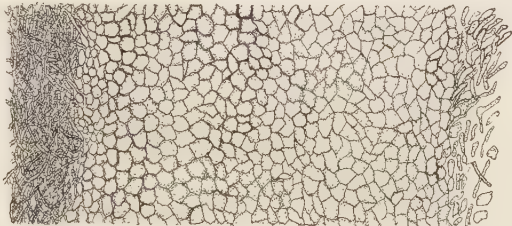
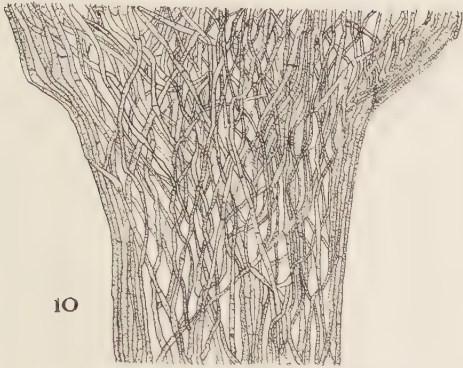


Fig. 12. A small section of the periphery of the sterile base of *Lycoperdon pulcherrimum* showing the parallel arrangement of the outermost hyphae.

Fig. 13. A diagrammatic cross section of a young fruit body of *Lycoperdon Wrightii* showing the relative thickness of the exoperidium.

Fig. 14. A detailed drawing showing the layers of the peridia of *Lycoperdon Wrightii* before the fissuring has begun. Note the thickness of the pseudoparenchymatous layer, and the loose connection of this layer to the endoperidium. This accounts for the splitting here. Compare with *Bovista plumbea*.

Fig. 15. A section of the exoperidium of *Lycoperdon pyriforme*, illustrating the manner in which the exoperidium becomes fissured, and how the outer cells become loosened and eventually fall away.

